Age-related changes in blood capillary endothelium of human dental pulp: an ultrastructural study

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Abstract

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Aim To describe the ultrastructural changes that occur in pulpal blood capillaries as a result of ageing.

Methodology Thirty samples of healthy dental pulps were obtained from functional human permanent teeth. Two age groups were examined: young (10–17 years) and old (>60 years). The teeth were extracted under local anaesthesia using mepivacaine without adrenaline (Scandonest 3%, Septodont, Saint-Maur des Fossés, France) and split longitudinally in a bench press. The pulps were gently removed, immersed in fixative solution, sectioned and processed by conventional transmission electron microscopic techniques. Micrographs were taken from the endothelium, and the whole capillary area of each vessel was examined.

Results In young pulps, the endothelial cell layer was characterized by the presence of numerous pinocytotic vesicles and microvesicles, RER cisterns, free ribosomes, a small Golgi complex, centrioles, microtubules, microfilaments and mitochondria. In the endothelial cell cytoplasm of older pulpal vessels, pinocytotic vesicles and microvesicles, as well as microfilaments, were more numerous. In addition, lipid-like vacuoles, monogranular glycogen granules and extensive Golgi complexes with dilated cisterns were also present. Weibel-Palade bodies were observed in both age groups without showing variations related with age.

Conclusions The results obtained in capillaries of aged pulpal tissue suggest that the endothelium experiences morphological changes that could be associated with advancing age.

Keywords: ageing, blood capillaries, dental pulp, endothelium, ultrastructure.

Introduction

The dental pulp is a loose connective tissue contained within a central cavity surrounded by an avascular hard tissue case. Nevertheless, the pulp receives a rich vascular supply. The blood vessels enter the pulp space via the apical foramen in association with the sensory and sympathetic nerve bundles. Additional blood supply is received through lateral canals. Arterioles and venules are arranged axially in the pulp with capillary loops extending out towards the dentine. In the coronal pulp, the capillary network is denser and more regular than in the root pulp (Geiger 1992). According to these morphological characteristics, it is evident that the vascular capillaries constitute a predominant part of the vascular supply of the pulp (Vongsavan & Matthews 1992, Matthews & Andrew 1995).

The dental pulp, like other body tissues, undergoes age-related changes. These changes are difficult to differentiate from physiological, defensive and pathological irritant-induced changes (Quigley 1971, Morse 1991). In some light microscopic investigations, impacted teeth have been used in order to eliminate the influence of the environmental effects prevalent in the oral cavity (Nitzan *et al*. 1986). However, because impacted teeth never erupt, they do not act as functioning teeth and

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any change detected may not reflect true physiological conditions (Morse 1991).

Age-related changes of the dental pulp have been extensively studied. As tooth gets older, pulp size and volume decrease due to the normal process of continuous apposition of secondary dentine. This tends to narrow the originally wide-open root apex (Domine & Holz 1991, Solheim 1992, Morse *et al.* 1993). Consequently, the vascular, lymphatic and nerve supply is compromised (Ketterl 1983, Morse 1991, Saad 1997). Although it has never been determined, this age-related interference in circulation and innervation may be the first step in dental pulp ageing (Morse 1991). Other age-related changes in the dental pulp include degeneration of odontoblasts, decrease in size and number of fibroblasts and increase in cross-linkages and number of mature collagen fibres, lipid infiltration and calcifications (Ketterl 1983, Morse 1991, Saad 1997).

Some investigations carried out under scanning electron microscopy have revealed the existence of a three-layer vascular network in the superficial layer of the young pulp: a terminal capillary network in the odontoblast layer, a capillary network formed with pre- and postcapillaries and a venular network. With advancing age, these characteristic three-layer vascular networks are substituted by a single, coarse, terminal network which converges directly with the main venules (Kishi *et al.* 1989, Takahashi 1990). Decreased circulation with age reduces the healing capacity of the pulp which, to some extent, also decreases the pulpal response to therapeutic agents. Thereafter, as teeth get older, the physiological and histological changes that take place in the pulp must be considered when dental treatment is being planned (Galán 1990, Burke & Samarawickrama 1995, Walton 1997).

Because of these morphological and functional characteristics, the dental pulp is an interesting model for the study of age-related changes in the microvascular system. The fine structure of the dental pulp blood vessels has been studied by transmission electron microscopy in both human (Dahl & Mjör 1973, Rapp *et al.* 1977, Carlile *et al.* 2000) and animal subjects (Corpron *et al.* 1973; 1974, Geiger 1992, Tabata *et al.* 1998). However, most of these investigations did not describe the changes that take place with ageing on vascular endothelium, which have a principal role in nutrient release, metabolite exchange and removal of waste products. The purpose of this study was to compare the possible ultrastructural modifications occurring in the endothelial wall cells of blood capillaries in aged compared to younger pulps.

Materials and methods

Dental pulp samples were obtained from human permanent teeth extracted for orthodontic or prosthetic reasons from patients undergoing treatment in the Clinic Areas of the Faculty of Dentistry, University of Zulia, Venezuela. Two age groups were studied: young-aged subjects (10–17 years) and advanced-aged subjects (>60 years). A total of 15 pulp samples were collected for each group. Only functional single-rooted teeth (incisors, canines and premolars) with no clinical or radiographic evidence of caries, periodontal disease or restorations were considered for this investigation. The teeth were extracted under local anaesthesia using mepivacaine without adrenaline (Scandonest 3%, Septodont, Saint-Maur des Fosses, France). Immediately after extraction, the teeth were split longitudinally in an especially adapted bench press and placed in 4% glutaraldehyde (Spi supplies, West Chester, PA, USA) in sodium cacodylate buffer (0.1 M; pH 7.4) for 30 min at 4 °C. The pulps were gently removed, sliced into small fragments and reimmersed in a fresh, similar solution for 1.5 h, followed by secondary fixation in 1% osmium tetroxide (EMS, Forth Washington, PA, USA) in cacodylate buffer for 1 h at 4 °C. Afterwards, the pulps were rinsed for 5–10 min in a buffer similar to that used in the fixation solution, dehydrated in increasing concentrations of ethanol and embedded in Araldite (Spi supplies, West Chester, PA, USA). For proper orientation of the electron microscopic study, thick sections of approximately 0.1–1 μm were stained with toluidine blue and examined with a photomicroscope (ZEISS, Oberkochen, Germany). Ultrathin sections (80 nm) obtained with a Porter-Blum ultramicrotome were stained with uranyl acetate (EMS, Forth Washington, PA, USA) and lead citrate (Spi supplies, West Chester, PA, USA) and observed in a transmission electron microscope (JEOL, Tokyo, Japan). Observations were made using intermediate magnifications ranging from ×20 000 to ×50 000. Micrographs taken from the endothelium and the total capillary area (luminal area plus endothelial cell area) of each blood vessel were examined.

Results

The blood capillaries obtained from pulpal tissue samples taken from young subjects were mainly of continuous type with regular thickness (Fig. 1a). In the odontoblastic layer, some capillaries were fenestrated. The capillaries presented a regular endothelial cell layer

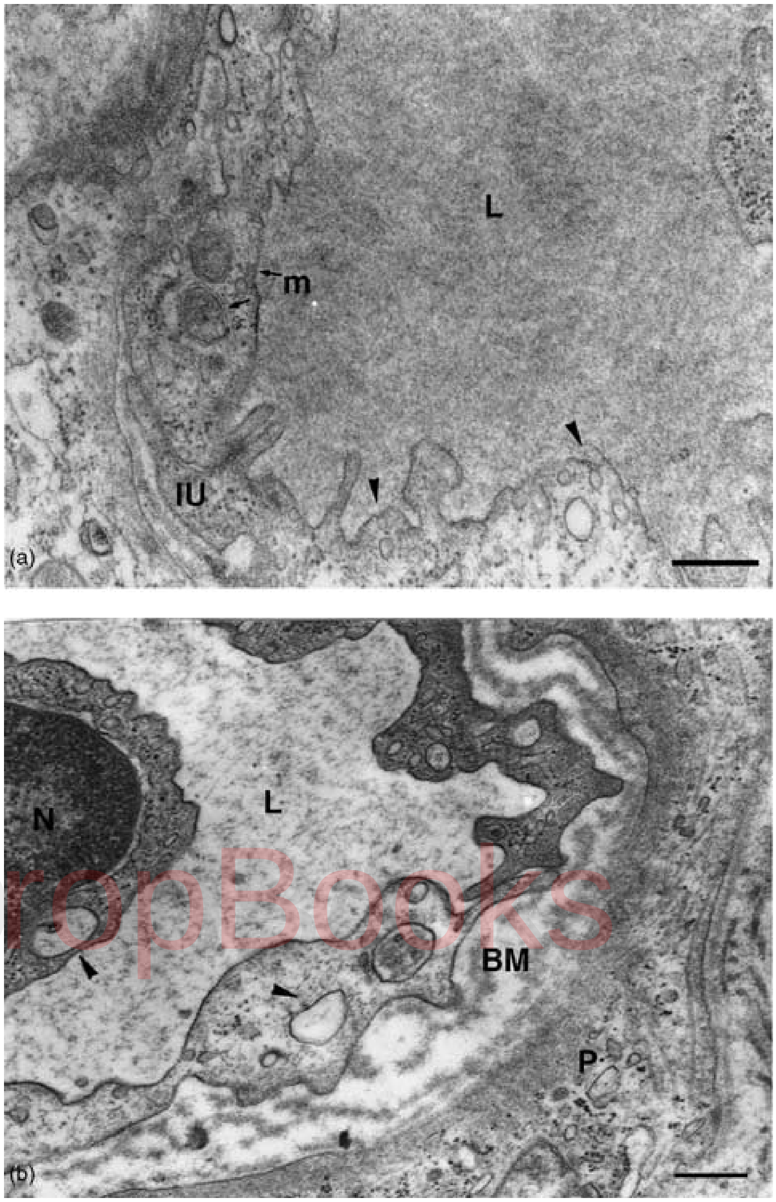

Figure 1 Electron micrographs of transverse sections of blood capillaries of the dental pulp. (a) Pulpal capillary from a young subject. The endothelium shows uniform thickness. Some pinocytic vesicles (arrowheads) and free ribosomes appear in the cytoplasm. m, mitochondria; IU, interendothelial union; L, vascular lumen. ×39 500. Bar = 300 nm. (b) Pulpal capillary from an aged subject. The endothelial cell has an irregular thickness. The cell nucleus (N) and cytoplasm appear denser than normal. Some vacuoles (arrowheads) are present in the cytoplasm. The basement membrane (BM) is discontinuous and electrondense. L, vascular lumen; P, pericyte cell. ×34 000. Bar = 300 nm.

in which specialized union complexes joined each cell. Some complexes had a short course (Fig. 2a) whilst others had a long and tortuous course (Fig. 2b). Ribosomes, mitochondria, rough endoplasmic reticulum (RER) cisterns, Golgi complexes, multivesicular bodies, microtubules and microfilaments were also detectable inside the endothelial cells (Fig. 2c). Weibel-Palade bodies appeared in rows or grouped near the intercellular adjoining area (Fig. 2d). Pinocytotic and micropinocytotic vesicles were present in the cytoplasm of the endothelial cells, principally along the abluminal side of the cellular membrane (Fig. 3a).

The blood capillaries were surrounded by a continuous basement membrane. Cytoplasmic components of perivascular cells were observed embedded in the basement membrane. Longitudinal and transverse sections of collagen fibres appeared as part of the pulpal connective tissue (Fig. 3a).

In aged dental pulps, only continuous capillaries with irregular thickness were observed (Fig. 1b). The capillary endothelium frequently showed intercellular adjoining structures with a prolonged course. The endothelial cell cytoplasm showed differences which were striking as compared to those obtained from younger individuals.

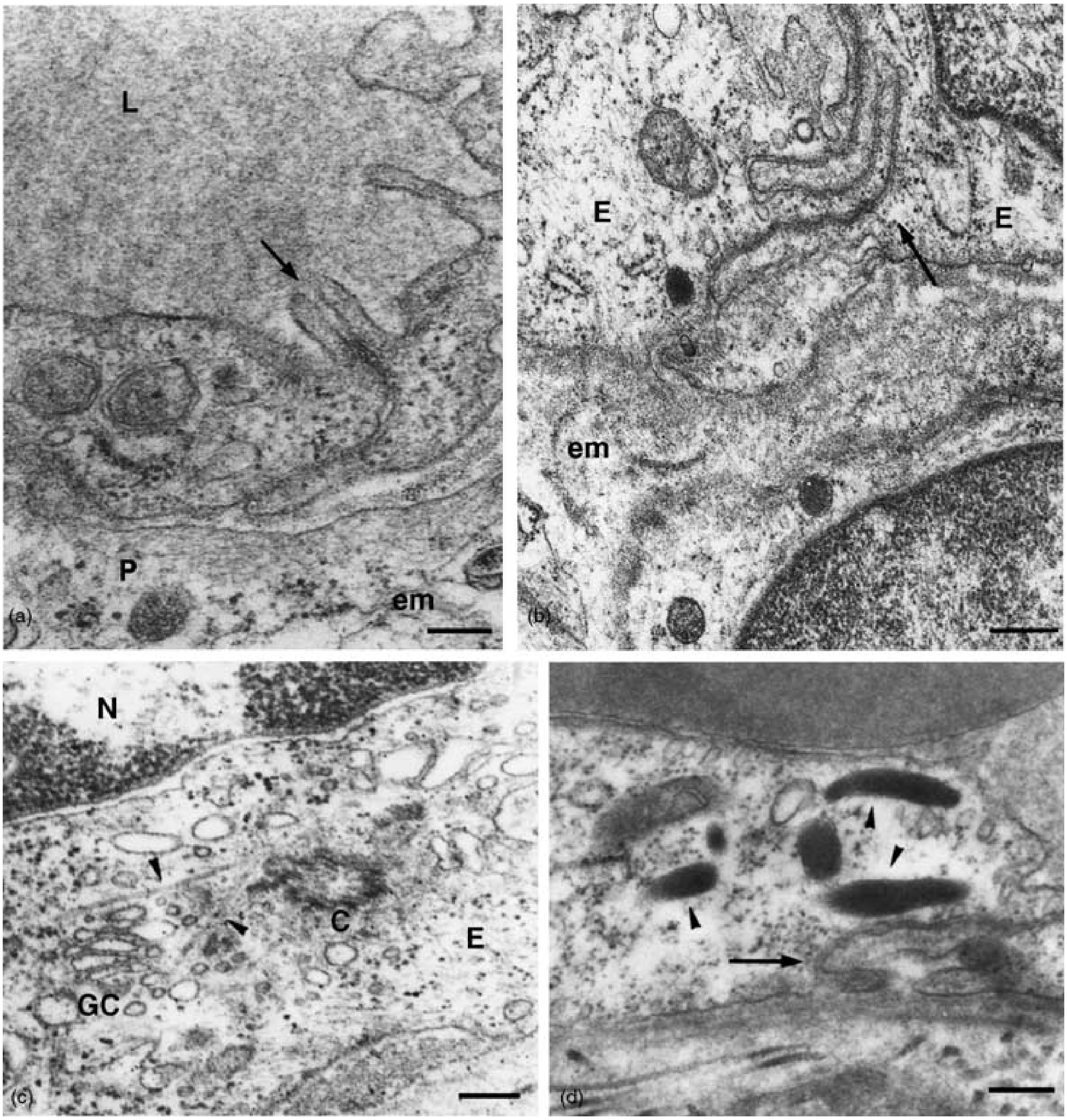

Figure 2 Electron micrographs of blood capillary walls from dental pulps of young subjects. (a) Interendothelial union complex with a short trajectory (arrow). A pericyte cell (P) is observed in the extracellular matrix (em). L, vascular lumen. ×75 000. Bar = 140 nm. (b) Intercellular union between two endothelial cells (E) with extensive and tortuous trajectory (arrow). em, extracellular matrix. ×44 000. Bar = 230 nm. (c) Some cytoplasmic organelles in the endothelial cell (E): a Golgi complex (GC), centriole (C), microtubules (arrowheads) and microfilaments. N, cell nucleus. ×56 500. Bar = 180 nm. (d) Weibel-Palade bodies (arrowheads) are grouped notably towards the area of the interendothelial union (arrow) between two adjacent cells. ×50 500. Bar = 200 nm.

In particular, pinocytotic and micropinocytotic vesicles increased towards the abluminal side of the cellular membrane (Fig. 3b), but they were clearly more numerous than in the younger tissues. Luminal vesicles with larger diameters were less numerous. Generally, microfilaments made contact with pinocytic vesicles and microvesicles at the level of the abluminal side of the cellular membrane (Fig. 4a).

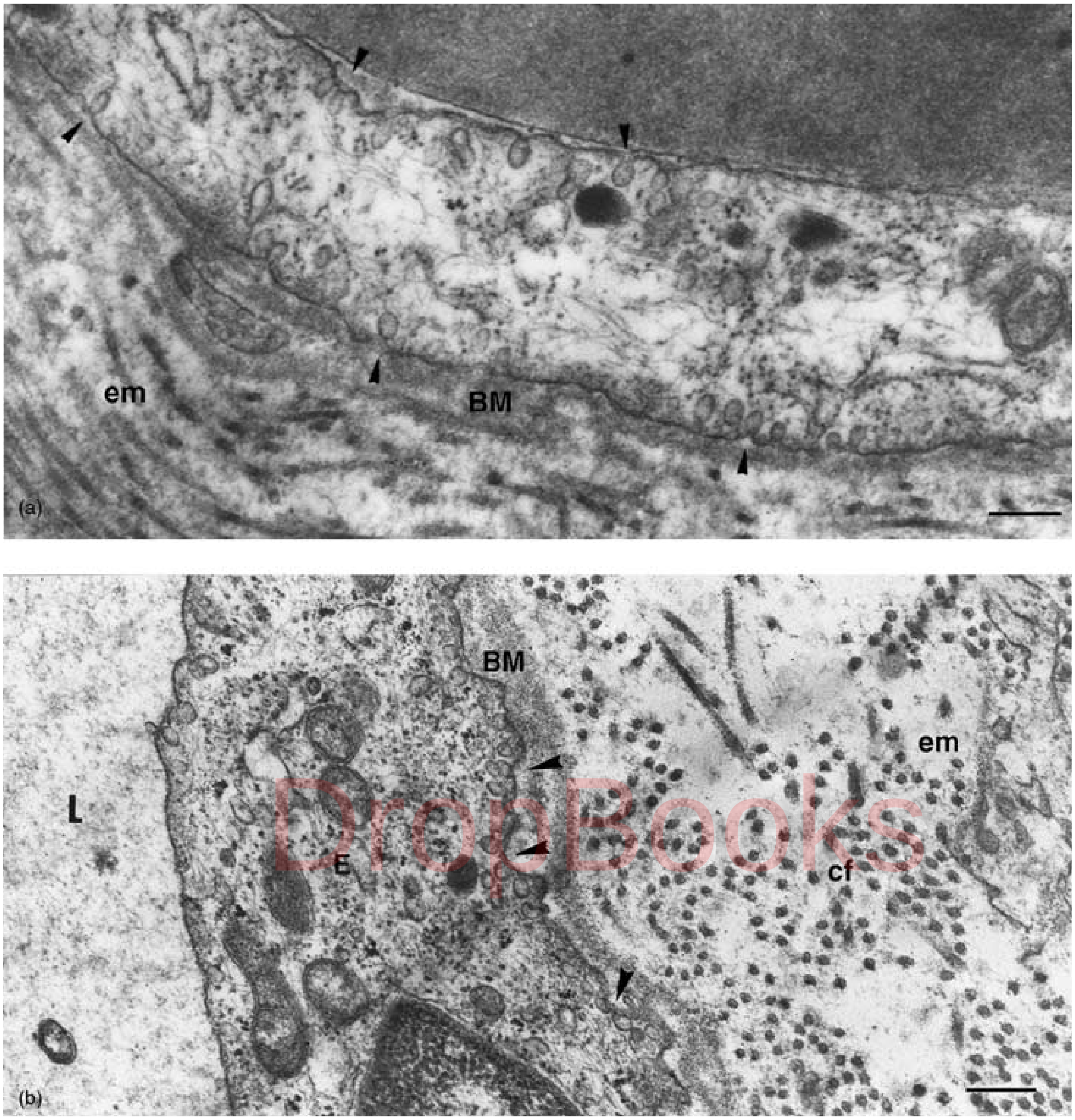

Figure 3 Electron micrographs of the transverse section of endothelial cells and perivascular area. (a) Pulpal capillary from a young subject. Open pinocytotic vesicles (arrowheads) are disposed in the luminal and abluminal sides of the cytoplasmic membrane. The basement membrane (BM) appears continuous and electrondense. em, extracellular matrix. ×50 500. Bar = 200 nm. (b) Pulpal capillary from an aged subject. More numerous pinocytotic vesicles and microvesicles (arrowheads) are present in the abluminal side of the membrane than in the luminal one. The basement membrane (BM) is more electrondense. Abundant transverse collagen fibres (cf) are observed in the extracellular matrix (em). L, vascular lumen; E, endothelial cell. ×44 000. Bar = 230 nm.

Another remarkable cytoplasmic variation was the presence of an extensive Golgi complex with dilated cisterns (Fig. 4b). Spherical lipid-like droplets (Fig. 4c) and monogranular glycogen granules (Fig. 4d) were also present. In some sections, abundant microfilaments formed a dense network in the cytoplasm, sometimes being the predominant cytoplasmic component (Fig. 4d). In addition, ribosomes, RER cisterns, multivesicular bodies and Weibel-Palade bodies were observed in the cytoplasm of the aged endothelial cells.

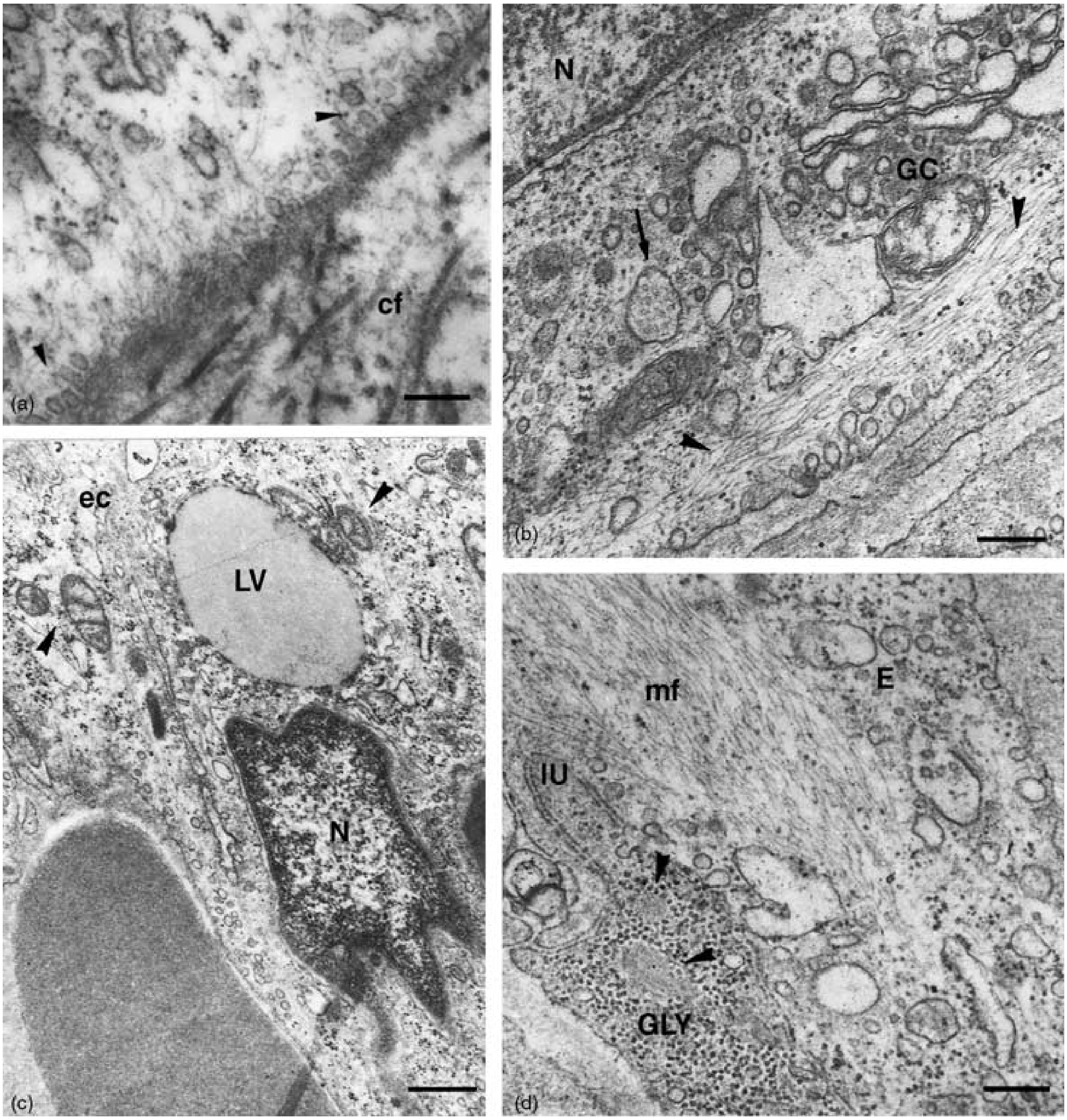

Figure 4 Electron micrographs of the endothelial wall of blood capillaries from dental pulp of aged subjects. (a) Dense microfilaments make contact with abluminal vesicles and microvesicles (arrowheads). Collagen fibres (cf) are present in the extracellular matrix. ×50 500. Bar = 200 nm. (b) Numerous microfilaments (arrowheads), some multivesicular bodies (arrow) and an extensive Golgi complex (GC) with dilated cisterns are noted in the cytoplasm of these cells. ×50 500. Bar = 200 nm. (c) A large lipid-like vacuole (LV) is present in the endothelial cytoplasm (ec). Some mitochondria (arrowheads) are observed. N, cellular nucleus. ×25 000. Bar = 400 nm. (d) Large amounts of monogranular glycogen granules (GLY) are noted in the cytoplasm of the endothelial cell (E). These granules mainly accumulate in the interendothelial union zone (IU). Some Weibel-Palade bodies (arrowheads) are surrounded by glycogen granules. Numerous microfilaments (mf) are present. ×50 500. Bar = 200 nm.

In some sections, the basement membrane was discontinuous and denser than in young pulp vessels. Collagen fibres were observed forming the fibrous component of the perivascular tissue (Fig. 3b). In other sections, the basement membrane appeared thicker and more homogeneous than normal.

Discussion

Due to its structural characteristics, the dental pulp is ideal for the study of possible morphological changes experienced by the blood microcirculation during ageing. Our research on human dental pulps, carried out in teeth extracted from young and aged individuals, allow some interesting observations.

In the pulps obtained from young subjects, the endothelial wall of blood capillaries was mainly continuous. However, a small proportion of fenestrated capillaries was localized in the vicinity of odontoblasts. These observations have been previously reported in both human (Rapp *et al.* 1977, Carlile *et al.* 2000) and animal specimens (Corpron *et al.* 1973; 1974, Oshima & Yoshida 1992). We also share their interpretation that this disposition permits a rapid transfer of nutrients towards the odontoblasts, the cells which are specialized in the production of dentine.

It has been demonstrated that under physiological conditions, vesicles and microvesicles of pinocytic origin are formed on the luminal side of the pulpal capillary endothelium and that they empty their content across the abluminal membrane towards the extracapillary space. The presence of pinocytotic vesicles mainly along the abluminal membrane has been previously reported in the dental pulp of young subjects (Rapp *et al.* 1977). In this investigation, an increase in the quantity of abluminal vesicles and microvesicles in the pulpal tissue obtained from aged subjects was observed. This finding supports the results obtained by Schellini *et al.* (1997) in which the pinocytotic vesicles were more numerous, mainly near the outer plasma membrane of retinal capillaries of aged rats. These investigators suggested that intense transvesicular transport is responsible for the removal of metabolites from the interstitium of the retina. Therefore, the increased transendothelial transport evidenced in aged vessels would contribute to the drainage of waste products generated in the subendothelial space.

The interactions between the cellular membrane and the cortical actin cytoskeleton are involved in endothelial cell metabolism, motility and permeability (Iijima *et al.* 1991, DuBose & Haugland 1993). In the endothelial cells of aged pulps, microfilaments, forming a dense arrangement which makes contact with the surface of the membranes that limit vesicles and microvesicles, were observed as described in the endothelium of other tissues such as the brain (Castejón 1984). When the vascular brain permeability is increased, as in traumatic cerebral oedema, components of the cytoskeleton participate in order to accelerate the vesicular and vacuolar transport through the endothelium. In this study, microfilaments present within the cytoskeleton facilitated the increased transendothelial transport.

According to Simionescu (cited by Castejón 1984), the Golgi vesicles constitute the structural vectors for the recycling of membranes. In the capillary endothelium of aged dental pulp, an extensive Golgi complex with dilated cisterns has been identified. It can be postulated (Castejón 1984) that when the vesicular and vacuolar transport through the endothelium is increased, great quantities of membranes derived from the Golgi complex are probably inserted in the luminal plasma membrane in order to provide new membranes. This sustained overfunction would induce the hypertrophy of the organelle.

Another remarkable finding in the endothelium of blood vessels of aged pulp tissue was the presence of cytoplasmic accumulations which have been associated previously with ageing. First, lipid-like vacuoles were present in the cytoplasm of capillary endothelial cell. Morse (1991) reported that the fat droplet deposit is the first observable change due to ageing in odontoblasts, endothelial cell and other pulpal cells. Hence, monoglicerides, diglicerides, phospholipids, lipoproteins, cholesterol and some of their esters have been isolated from such deposits.

On the other hand, large amounts of monogranular glycogen granules were also present within the endothelial cytoplasm. These deposits have been described by Schellini *et al.* (1997) in the endothelial and pericytial cells of the retinal capillaries of aged rats and the same have been considered as a morphological sign of ageing.

The Weibel-Palade bodies are cytoplasmic organelles present in the endothelial cells of vertebrates. It has been demonstrated that they contain von Willebrand factor and P-selectin and that they release these adhesive molecules during the inflammatory processes (Marchetti 1996). These organelles have been identified in healthy and inflamed human dental pulp in the endothelium of blood (Jacoby *et al.* 1991, Carlile *et al.* 2000) and lymphatic capillaries (Marchetti & Piacentini 1990, Marchetti *et al.* 1990; 1991; 1992, Marchetti 1996). Jacoby *et al.* (1991) demonstrated that the endothelial cells of pulpal blood capillaries were actively committed in the synthesis of the von Willebrand factor, which is increased during tissue injury.

On the other hand, Carlile *et al.* (2000) reported that Weibel-Palade bodies were located without an obvious pattern of orientation throughout the endothelial cytoplasm of blood capillaries of human dental pulp. However, it shows herein that these organelles accumulate with preference towards the interendothelial zone in blood capillaries of both young and aged dental pulps. Similar findings were obtained by Marchetti & Piacentini (1990) in the lymphatic capillaries of healthy pulpal tissue of young teeth. This would appear to be the first description of this Weibel-Palade distribution in the blood capillaries of human dental pulp. It remains necessary to clarify whether this finding has a functional significance and if variations are experienced due to different physiological or pathological conditions in pulpal tissue. Based on the findings of this study, it can be postulated that the Weibel-Palade bodies apparently do not show either qualitative or quantitative variations that could be related with the ageing in an individual.

Further investigations must be considered regarding other structures in dental pulp such as innervation and lymphatic supply as well as odontoblasts, fibroblasts and immune cells. This would permit the development of therapeutics, successful agents and strategies for the geriatric patient.

Conclusions

The results contribute to the understanding of biological conditions of the microcirculation in aged dental pulp tissue. With the advancement of age, the capillary endothelium experiences morphological changes such as an increased transendothelial transport, cytoskeletal changes, a hypertrophic Golgi complex and cytoplasmic deposits.

The existence of extensive microvascular interconnections amongst the periodontal ligament, gingival tissue and the dental pulp has been demonstrated. Because the blood vessels perform specific functions in each of these tissues, it would be of interest to compare the ultrastructure of their microvasculature in old age when the vitality of the dental pulp and periodontal tissue is fundamental for preservation of the dentition.

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